

Evaluation of analgesic effect and absorption of buprenorphine after buccal administration in cats with oral disease

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Complete List of Authors:	Stathopoulou, Thaleia- Rengina; Royal Veterinary College Clinical Services Division, Kouki, Maria; Aristotle University of Thessaloniki School of Veterinary Medicine Pypendop, Bruno; School of Veterinary Medicine, University of California, Surgical and Radiological Sciences Johnston, Atholl; St George's, University of London, Pharmacology and Toxicology Papadimitriou, Serafeim; Aristotle University of Thessaloniki School of Veterinary Medicine Pelligand, Ludovic; Royal Veterinary college, Clinical Services and Sciences,
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Abstract:	<p>Objectives: To evaluate the analgesic effect and the absorption of buprenorphine after buccal administration in cats with oral disease.</p> <p>Methods: Six adult client-owned cats with chronic gingivostomatitis (weighing 5.1kg +/- 1.1kg) were recruited for a randomized, prospective, blinded, saline controlled crossover study. Pain scores, dental examination, stomatitis score and buccal pH measurement were conducted on day 1 under sedation in all cats. On day 2, animals were randomized in two groups and administered one of the two treatments buccally (group A received buprenorphine (0.02mg/ kg and group B received 0.9% saline) and vice versa on day 3. Pain scores and food consumption were measured at 30, 90 and 360 mins after the administration of buprenorphine. Blood samples were taken at the same times and plasma buprenorphine concentration were measured by liquid chromatography- mass spectrometry. Data were statistically analysed as non-parametric and level of significance was set as $P < 0.05$.</p> <p>Results: There were no major side effects after buprenorphine administration. Buccal pH values ranged between 8.5-9.1 and stomatitis disease activity index 10-22 (17.8 +/- 4.5) with the scale ranging from 0-30. The maximum buprenorphine plasma concentration (14.8 ng/ ml) was observed 30 minutes after administration and there was low interindividual variability. There was a significant difference between baseline pain scores compared to pain scores after buprenorphine ($P < 0.05$) and between the saline and buprenorphine group at 30 mins ($p = 0.04$) and 90 mins ($P = 0.04$). There was also a significant effect of stomatitis index on pain score. Regarding the pharmacokinetic parameters, cats with stomatitis showed lower bioavailability and shorter absorption half-life after buccal</p>

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Evaluation of analgesic effect and absorption of buprenorphine after buccal administration in cats with oral disease

Thaleia- Rengina Stathopoulou,¹ Maria Kouki,² Bruno Pypendop,³ Atholl Johnston,⁴ Serafeim Papadimitriou,² Ludovic Pelligand¹

¹ Clinical Services and Sciences, Royal Veterinary College, , Hawkshead Lane, Hatfield, UK AL97TA

² Aristotle University of Thessaloniki School of Veterinary Medicine, Thessaloniki GR

³ Surgical and Radiological Science, School of Veterinary Medicine, University of California, One Shields Avenue, Davis, CA, USA 95616

⁴ Forensic Pharmacology and Toxicology, St George's, University of London, Cranmer Terrace, London SW17 0RE

Keywords: feline, buprenorphine, gingivostomatitis, transmucosal absorption, analgesia

Corresponding author: Thaleia- Rengina Stathopoulou DVM, MRCVS, MVetMed, Royal Veterinary College, Clinical Services and Sciences, Hawkshead Lane, Hatfield, UK AL97TA

Email: tstathopoulou@rvc.ac.uk

24 Abstract

25 **Objectives:** To evaluate the analgesic effect and the absorption of buprenorphine after buccal
26 administration in cats with oral disease.

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28 recruited for a randomised, prospective, blinded, saline controlled crossover study. Pain scores,
29 dental examination, stomatitis score and buccal pH measurement were conducted on day 1 under
30 sedation in all cats. On day 2, animals were randomized in two groups and administered one of the
31 two treatments buccally (group A received buprenorphine (0.02mg/ kg and group B received 0.9%
32 saline) and vice versa on day 3. Pain scores and food consumption were measured at 30, 90 and 360
33 mins after the administration of buprenorphine. Blood samples were taken at the same times and
34 plasma buprenorphine concentration were measured by liquid chromatography- mass spectrometry.
35 Data were statistically analysed as non-parametric and level of significance was set as $P < 0.05$.

36 **Results:** There were no major side effects after buprenorphine administration. Buccal pH values
37 ranged between 8.5-9.1 and stomatitis disease activity index 10-22 (17.8 +/- 4.5) with the scale ranging
38 from 0-30. The maximum buprenorphine plasma concentration (14.8 ng/ ml) was observed 30 minutes
39 after administration and there was low interindividual variability. There was a significant difference
40 between baseline pain scores compared to pain scores after buprenorphine ($P < 0.05$) and between the
41 saline and buprenorphine group at 30 mins ($p = 0.04$) and 90 mins ($P = 0.04$). There was also a significant
42 effect of stomatitis index on pain score. Regarding the pharmacokinetic parameters, cats with
43 stomatitis showed lower bioavailability and shorter absorption half-life after buccal administration of
44 buprenorphine compared to normal cats.

45 **Conclusion and clinical relevance:** Buccal administration of buprenorphine in cats with oral disease
46 produces an analgesic effect and low interindividual variability. Buprenorphine administered
47 buccally can be incorporated in the multimodal analgesia plan of cats with gingivostomatitis.

48 Introduction

49 Pain management is the cornerstone of veterinary practice and constitutes not only a professional
50 obligation but also a way to enhance animals' quality of life. In the recent years, there has been
51 increased interest into pain assessment and management in cats that have been historically
52 undertreated for pain compared to other species.¹⁻³

53 Opioids play an important role in the multimodal approach to pain management in cats with
54 buprenorphine being one of the drugs most widely used.⁴ Buprenorphine, a highly lipophilic semi
55 synthetic partial agonist at μ (mu) opioid receptors, is considered a unique drug with complex
56 pharmacology.⁵ It is the most commonly used opioid in small animal practice in the UK,¹ being also
57 widely used in the vast majority of continental Europe, Australia and South Africa.^{2, 6} Common
58 morphine and hydromorphone side effects such as nausea, vomiting and salivation are rarely seen
59 after buprenorphine. This advantage, alongside with its efficacy and long duration of action⁷⁻⁹ justify
60 its popularity.

61 In feline patients, studies have proven that the buccal route of administration (OTM) of
62 buprenorphine shows a bioavailability similar to the intravenous (IV) and intramuscular (IM)
63 routes.¹⁰⁻¹² According to Robertson et al (2005),¹⁰ the analgesia provided by the buccal administration
64 is comparable to the one of alternative routes. However, among others the study from Giordano et al.
65 (2010)¹³ demonstrated inferior analgesic effect of the buccal route compared to IV and IM after
66 ovariectomy and Santos et al¹⁴ found less sedative effect after buccal administration of
67 dexmedetomidine and buprenorphine compared to IM route.

68 The systemic absorption of buprenorphine, after buccal administration depends on the mucosal pH.
69 Buprenorphine is a weak base pKa (8.24) and therefore an alkaline environment, such as the cat's oral
70 cavity with pH between 8 and 9, favours its unionised form and enhances its bioavailability by
71 avoiding the first pass elimination.

72 The blood sampling site has also, an impact on buprenorphine concentration–time profile. Following
73 buccal administration in cats, venous blood sampling from a jugular site is not an acceptable
74 substitute for arterial blood sampling,¹⁵ as the perfusion of the oral mucosa drains from the same vein
75 resulting in overestimation of drug's systemic availability. The above can explain the high
76 bioavailability of buprenorphine (116%) found in previous studies¹⁰ following buccal administration
77 as external jugular was used for sampling.

78 Severe inflammation of the oral cavity, described with the term gingivostomatitis¹⁶ is a multifactorial
79 disease often seen in feline patients and it can be a chronic, devastating and painful condition. The
80 exact aetiology of the condition is unknown, with environmental factors, bacterial and viral infection
81 to being most often implicated,¹⁷ though neoplastic, autoimmune, developmental and congenital
82 conditions can be recognised as co-factors as well. Clinical signs include oral pain, halitosis,
83 dysphagia, anorexia and weight loss, while some cats are euthanized because of poor quality of life.¹⁸
84 Treatment of gingivostomatitis is mainly symptomatic and involves antibiotics, corticosteroids,
85 opioids, non-steroidal anti- inflammatory agents (NSAIDs), laser thermoablation, cyclosporine, oral
86 surgery and tonsillectomy. Plasmapheresis, human immunoglobulin and feline interferon omega have
87 also been used.¹⁹

88 We designed a saline-controlled crossover efficacy and pharmacokinetic study in cats with
89 gingivostomatitis to assess whether the presence of oral inflammation in the oral cavity affected the
90 rate of oral transmucosal absorption, the overall systemic uptake and the analgesic efficacy of
91 buprenorphine. Our alternative hypothesis was that there would be difference in analgesia between
92 the buprenorphine and saline groups after buccal administration, with buprenorphine providing
93 superior analgesia. The prevalence of feline gingivostomatitis in the UK is 0.7%, but appears to be
94 much higher (13.1%) in studies in United States and Southern Europe.¹⁸ Due to the higher prevalence
95 of oral diseases in Southern Europe we recruited patients at the Aristotle University (Greece).²⁰

96

97 **Materials and methods**

98 The study was designed as a randomised, prospective, saline-controlled, blinded crossover study. The
99 design is summarised in Figure 1. Ethics approval was granted by the Aristotle University of
100 Thessaloniki, Greece and written owner consent was obtained for this clinical trial.

101 Six client owned adult cats, ASA (American Society of Anaesthesiologists) physical status I or II, with
102 evidence of oral inflammation were included in the study. No abnormal finding other than signs of
103 oral inflammation was detected during physical examination. The cats had not received any opioids
104 five days prior their arrival. Concurrent NSAIDs and/or antibiotics course were not exclusion criteria.

105 Allocation of the first treatment was randomised by the means of sealed envelopes containing the
106 number of each cat. The first three chosen by a blinded investigator were assigned to group A and the
107 rest to group B.

108 On day 1, physical examination was performed and baseline pain scores were recorded, according to
109 a modified BOTUCATU pain scale²¹ (range from 0 to 27, Appendix 1). All cats were, subsequently,
110 sedated with 0.02mg/kg medetomidine intramuscularly (IM) (Sedastart, Animalcare). During
111 sedation, oral pH was measured with pH stripes (Simplex Health), oral lesions were staged and
112 mapped using a dental examination form and stomatitis disease activity index²² (Appendix 2, 3). An
113 intravenous peripheral catheter (22G, 25mm. Jelco, Smiths Medical) was placed in a cephalic vein to
114 facilitate blood sampling and to decrease any additional discomfort for the patients. Sedation was
115 reversed with 0.05 mg/kg of atipamesole (Sedastop, Animalcare) IM. The catheters were flushed every
116 4 hours with 2ml of heparinised saline to secure their potency and a light bandage was placed for
117 protection.

118 On day 2, the cats from group A received 0.02 mg/kg of buprenorphine (group BUP, Buprecare,
119 Animalcare) by buccal route and group B received equal volume of 0.9% saline (group SAL,
120 Vetivex1, Dechra Animal Products) by the same route. Both treatments were administered with a 1 ml

syringe (B. Braun medical) in the right cheek pouch by the principal investigator (TS) that was blinded to treatment allocation. Cats were assessed for the presence of hypersalivation, mydriasis, grooming activity and food consumption (yes/no) 30, 90 and 360 minutes following the treatment administration. Pain assessments were performed by the same investigator at the same times using the same scale as before.

Blood samples were collected by the assessor (MK), who was aware of treatment allocation, 30, 90 and 360 mins after buprenorphine buccal administration, but not after saline administration. Following pain scoring, samples were taken from the cephalic catheter after 2 ml of blood were aspirated to ensure a non-diluted blood sample. One ml of blood was collected in potassium EDTA blood tubes (Vetlab). The samples were centrifuged (Centrifuge Heraeus -Christ GmbH Osterode, Harz Simplex, GE) for eight minutes at 4039g within 30minutes after collection. The plasma (0.5 to 0.7 mL) was separated and stored in -80 °C (Model 725, Thermo-Forma) in labelled Eppendorf tubes.

On day 3, the alternative treatment was administered, with group A receiving the 0.9% saline treatment and group B receiving 0.02 mg /kg buprenorphine buccally, and the same procedure as on day 2 was followed.

Plasma samples were shipped to the UK on dry ice and analysed by St Georges University in London. Plasma buprenorphine was measured using a validated liquid chromatography – tandem mass spectrometry method (LC/MS/MS),²³ initially validated in man. The method was revalidated for feline plasma and met standards for sensitivity, linearity, precision, accuracy and stability generally accepted in bioanalytical chemistry.²⁴ The lower limit of quantification of the assay was 0.025 ng/mL.

Population pharmacokinetic modelling was performed with Phoenix NMLE®, version 1.3, Certara (Princeton, NJ, USA). Briefly, a two-compartmental model was built to be simultaneously fitted to the plasma buprenorphine concentration-time data from the present study (sparse sampling) and those from a previously published study performed in healthy cats administered the same dose of buprenorphine intravenously and by the buccal route (rich sampling).²⁵ Full description of the joint

population PK model is provided in Appendix 4. The goal of including external IV and buccal route data in the PK model was to leverage information (clearances and volumes of distribution assumed to be distributed similarly in stomatitis and healthy cats) and increase the number of degree of freedom, as done in Pelligand et al.²⁶ This allowed the fitting of the most likely plasma concentration time-curve in sparsely sampled cats and the estimation of bioavailability and absorption rate constant in the study with stomatitis cats.

Statistical analysis

A commercially available programme was used for the statistical analysis (IBM SPSS Statistics 22). Data distribution was assessed for normality graphically and by the results of Kolmogorov -Smirnov statistic. Due to violation of the assumption of normality, the Wilcoxon matched -pairs signed rank test was used to compare pain scores obtained as baseline, after saline and after buprenorphine administration and at 30, 90 and 360 mins. The level of significance was set as $P < 0.05$. Pharmacokinetic parameters distributions were compared between cats with gingivostomatitis and normal cats²⁵ using the Mann-Whitney U-test.

Correlation analysis was used to describe the strength and the direction of the linear relationship between variables. Spearman Rank Order Correlation was used for non-parametric data testing of correlation between stomatitis activity index score and both pH and pain scores. Food consumption (yes/no) was tested at each time point with a Fisher's exact test.

Results

Six, client owned, adult cats were included in this clinical study, four male neutered and two female neutered. Their age ranged from 7 to 10 years (mean 9.1years) and their body weight ranged from 4 to 7 kg (mean 5.1kg). Two of the cats were receiving antibiotics and one of them was also receiving meloxicam for their stomatitis.

No adverse effects were noted in this study except hypersalivation in two of the cats after the administration of buprenorphine that resolved within minutes. All cats developed mydriasis within 5 minutes after the administration of buprenorphine, except in one cat in which this could not be evaluated due to bilateral enucleation. Mydriasis persisted for several hours after buprenorphine administration.

The oral pH values ranged from 8.5 to 9 and the stomatitis disease activity index ranged from 10 to 22 (mean 17.8+/- 4.5). Three of the cats had partial mouth extractions of the premolar and molar teeth and three had previously full mouth extractions. The positive correlation between the variables of pH and stomatitis disease index and pH was not significant ($P = 0.152$).

Food consumption evaluation was part of the total pain scores. At 30 mins, all cats in the buprenorphine group ate some wet food compared to 2 in the saline group ($P = 0.061$). At 90 minutes, cats treated with buprenorphine had a significantly higher chance to eat than with saline (6 cats for buprenorphine vs 1 saline, $P = 0.0152$). There was no difference at 360 minutes (2 cats for buprenorphine vs 3 cats for saline, $P = 0.54$). None of the cats started eating dry food at any time point.

Pain scores (figure 2) decreased significantly with buprenorphine (BUP) and saline (SAL) administration compared to baseline (BSL, $P = 0<.001$). When testing each time point, the pain scores for the BUP group were significantly lower than BSL at 30 mins ($P = 0.0007$) and 90 mins ($P=0.011$) and were significantly lower than SAL at 30 mins ($P=0.04$) and at 90mins ($P=0.04$), but not at 360 mins

($P=0.09$). Linear mixed model also revealed a significant effect of stomatitis effect of stomatitis index on pain score ($P=0.001$).

The time of maximum buprenorphine plasma concentrations in cats with gingivostomatitis was at the 30-mins blood sample when concentrations ranged from 274 to 1 621 ng/ mL. One cat (10-year female neutered 4.2kg cat treated with clindamycin, meloxicam, dental score 18) had a very high plasma concentration (84 979 ng/mL). This data point was excluded from the analysis on the basis that such high plasma concentrations were not reached even in early 1 and 3 minute samples after IV administration²² and is likely to result from contamination of the sample. The most likely buprenorphine plasma concentration-time plot for the cats with gingivostomatitis is shown in figure 3. For all parameters listed below, the inter-individual variability (IIV %) is reported immediately following each estimate where appropriate. Pharmacokinetic parameter (Table 1) estimates for clearance, intercompartmental clearance, volume of distribution of the central and peripheral compartment displayed low inter-individual variability even in a mixed group and were close to values previously reported.²⁵

The pharmacokinetic parameters are presented in Table 1 and described in Appendix 4.

Discussion

During this study, no side effects were identified, except hypersalivation in two cats. All cats, except the one that had bilateral enucleation, developed mydriasis.

There is a lack of evidence in veterinary literature on whether oral inflammation affects buccal pH values. The values of buccal pH in our study ranged between 8.5 and 9.1 and are relatively lower compared to Robertson's study¹⁰ (pH =9.0) but higher compared to Hedges's²⁵ (pH =8.0). A correlation between the buccal pH and the stomatitis disease activity index was not identified. An

214 increase in pH is associated with increased salivation in humans²⁷ due to an increase of sodium and
215 bicarbonate.²⁸ In cats, stomatitis is often related with signs of hypersalivation.¹⁶

216 Cats showed increased appetite at 30 and 90 mins after buprenorphine administration, which could
217 be due to additional analgesia or euphoria. An increase in food consumption is a rare manifestation of
218 pain in cats.²⁹ None of the cats ate dry food which could be due to insufficient pain relief or to
219 preference as cats were offered simultaneously wet and dry food. The influence of a hospital
220 environment should also be considered. Some cats remain unresponsive and passive in new
221 environments or can be hyperactive.^{30, 31} Increased food intake would be an important benefit,
222 considering that compromised nutrition is one of the most important problems encountered with
223 gingivostomatitis.³²

224 Pain scores following buprenorphine administration were lower than at baseline and following saline
225 administration. This can be attributed to pain relief as well as the euphoria produced by opioids. In
226 addition, local effect of buprenorphine needs to be considered since a study in humans found that
227 buprenorphine decreased the postoperative pain and increased the duration of analgesia when added
228 to the inferior alveolar nerve block for dental surgery, compared to intramuscular
229 administration.³³ The effect of stomatitis index on pain score was expected, as cats with more severe
230 stomatitis are expected to be more painful. Our alternative hypothesis that pain scores would be
231 lower following buprenorphine than following saline was confirmed, as there was a significant
232 difference at 30 and 90 mins. The plasma buprenorphine concentration at 360 mins may have been
233 inadequate to provide analgesia. In any case, the results may suggest that the duration of effect of
234 buprenorphine at the dose used may be shorter than previously reported.

235 The time of maximum plasma buprenorphine concentration was 30 minutes following administration
236 and pharmacokinetic analysis showed low interindividual variability with values close to those
237 obtained by Hedges et al²⁵ in cats with normal oral mucosa. Transmucosal drug absorption, though,
238 depends on many different factors like its concentration and the mucosal contact time.³⁴

Buprenorphine was administered in the cheek pouch but the degree of inflammation on the specific area could not be determined. Inflammation-induced vasodilation could have led to an earlier maximum concentration that we were unable to detect as our first blood sample was at 30 min. In addition, cats might have swallowed or spitted a portion of the drug, as they were sensitive in handling of the head and did not tolerate their mouth to be held closed after treatment. The formulation used in this study was a multi-dose vial (Buprecare, Animalcare,) containing 0.135% chlorocresol as a preservative and it is possible that the preservative free buprenorphine could be better tolerated.³⁵ The multi-dose vials are commonly used in practice due to cost effectiveness and easy usage and storage.

In our study, the mean absorption half-life of buprenorphine was longer compared to Hedges et al.,²⁵ which included normal cats. However, there was no significant difference in bioavailability, although the present study may have been underpowered to detect a difference. The difference in absorption rate could be due either to the different formulations of buprenorphine that were used in the two studies, to the actual modalities of administration or an effect of the higher pH.

The study had several limitations. The lack of a sensitive and validated pain scale for oral pain is a major limitation. UNESP-Botucatu scale is the only pain scoring system for cats with published data on reliability, validity and sensitivity²⁹ and we modified it for oral pain using the oral cavity as the painful reference point and the head and neck area as the surrounding tissues. We omitted the blood pressure measurement because it could be stressful and unreliable when repeated in frequent intervals. The maximum point of our pain scale was 27 instead of 30 in the original scale. The small sample size is another limitation that could have affected our statistical analysis. Furthermore, the use of historical data for modelling in lieu constitutes one more limitation, as is the use of data from another study that were obtained under different conditions and analysed using a different assay, despite that they were remodelled using the study population model. Finally, the values of buccal pH

were also obtained on day 1 after the administration of medetomidine that could have also affected the value, so we are not aware of the actual pH value on the time of buprenorphine administration.

Conclusion

Buccal administration of buprenorphine in cats with oral disease produces an analgesic effect and has low interindividual variability. Further studies are needed to elucidate the role of oral inflammation on buccal drug absorption in cats. Furthermore, considering that sublingual buprenorphine constitutes an effective treatment of chronic pain in humans ³⁶ and that subcutaneous buprenorphine prevented hyperalgesia in cats,³⁷ studies on the long-term use of buprenorphine by the buccal route in cats with chronic gingivostomatitis and the evaluation of the potential benefits and side effects would be of clinical interest.

Supplementary material

Appendix 1: UNESP-Botucatu Multidimensional Composite Pain Scale for assessing postoperative pain in cats, modified to assess oral pain.

Subscale 1: PAIN EXPRESSION (0 – 12)		
Miscellaneous behaviour	<p>Observe and mark the presence of the behaviours listed below</p> <p>A - The cat is laying down and quiet, but moving its tail</p> <p>B - The cat contracts and extends its thoracic limbs and/or contracts its neck muscles</p> <p>C - The cat's eyes are partially closed (eyes half closed)</p> <p>D - The cat licks and/or bites the surgical wound</p> <ul style="list-style-type: none"> • All above behaviours are absent • Presence of one of the above behaviours <input type="checkbox"/> Presence of two of the above behaviours <input type="checkbox"/> Presence of three or all of the above behaviours 	<p>A</p> <p>B</p> <p>C</p> <p>D</p> <p>0</p> <p>1</p> <p>2</p> <p>3</p>
Reaction to palpation of the area around the mouth cavity	<ul style="list-style-type: none"> • The cat does not react when the mouth is touched or pressed; • The cat does not react when the area around the mouth is touched, but does react when it is pressed. It may vocalize and/or try to bite • The cat reacts when the mouth is touched and when pressed. It may vocalize and/or try to bite • The cat reacts when the observer approaches the mouth. It may vocalize and/or try to bite <p>The cat does not allow palpation around mouth cavity</p>	<p>0</p> <p>1</p> <p>2</p> <p>3</p>
Reaction to palpation of the head	<ul style="list-style-type: none"> • The cat does not react when the head is touched • The cat does not react when the head and neck are touched, but does react when it is pressed. The neck is tense • The cat reacts when the head and neck are touched and when pressed. The neck is tense • The cat reacts when the observer approaches the head It may vocalize and/or try to bite <p>The cat does not allow palpation of the head and neck</p>	<p>0</p> <p>1</p> <p>2</p> <p>3</p>

vocalisation	• The cat is quiet, purring when stimulated, or miaows interacting with the observer, but does not growl, groan, or hiss	0
	• The cat purrs spontaneously (without being stimulated or handled by the observer)	1
	• The cat growls, howls, or hisses when handled by the observer (when its body position is changed by the observer)	2
	• The cat growls, howls, hisses spontaneously (without being stimulated or handled by the observer)	3

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Subscale 2: PSYCHOMOTOR CHANGE (0 – 12)		
posture	• The cat is in a natural posture with relaxed muscles (it moves normally)	0
	• The cat is in a natural posture but is tense (it moves little or is reluctant to move)	1
	• The cat is sitting or in sternal recumbency with its back arched and head down; or The cat is in dorso-lateral recumbency with its pelvic limbs extended or contracted	2
	□ The cat frequently alters its body position in an attempt to find a comfortable posture	3
comfort	• The cat is comfortable, awake or asleep, and interacts when stimulated (it interacts with the observer and/or is interested in its surroundings)	0
	• The cat is quiet and slightly receptive when stimulated (it interacts little with the observer and/or is not very interested in its surroundings)	1
	• The cat is quiet and “dissociated from the environment” (even when stimulated it does not interact with the observer and/or has no interest in its surroundings) The cat may be facing the back of the cage	2
	□ The cat is uncomfortable, restless (frequently changes its body position), and slightly receptive when stimulated or “dissociated from the environment” The cat may be facing the back of the cage	3
activity	• The cat moves normally (it immediately moves when the cage is opened; outside the cage it moves spontaneously when stimulated or handled)	0
	• The cat moves more than normal (inside the cage it moves continuously from side to side)	1
	• The cat is quieter than normal (it may hesitate to leave the cage and if removed from the cage tends to return, outside the cage it moves a little after stimulation or handling)	2
	□ The cat is reluctant to move (it may hesitate to leave the cage and if removed from the cage tends to return, outside the cage it does not move even when stimulated or handled)	3
attitude	Observe and mark the presence of the mental states listed below	
	A - Satisfied: The cat is alert and interested in its surroundings (explores its surroundings), friendly and interactive with the observer (plays and/or responds to stimuli) *The cat may initially interact with the observer through games to distract it from the pain. Carefully observe to distinguish between distraction and satisfaction games	A
	B - Uninterested: The cat does not interact with the observer (not interested by toys or plays a little; does not respond to calls or strokes from the observer) * In cats, which don't like to play, evaluate interaction with the observer by its response to calls and strokes	B
	C - Indifferent: The cat is not interested in its surroundings (it is not curious; it does not explore its surroundings) * The cat can initially be afraid to explore its surroundings. The observer needs to handle the cat and encourage it to move itself (take it out of the cage and/or change its body position)	C
	D - Anxious: The cat is frightened (it tries to hide or escape) or nervous (demonstrating impatience and growling, howling, or hissing when stroked and/or handled)	D

	E - Aggressive: The cat is aggressive (tries to bite or scratch when stroked or handled)	E
	<input type="checkbox"/> Presence of the mental state A	0
	<input type="checkbox"/> Presence of one of the mental states B, C, D, or E	1
	<input type="checkbox"/> Presence of two of the mental states B, C, D, or E	2
	<input type="checkbox"/> Presence of three or all of the mental states B, C, D, or E	3
Subscale 3: PHYSIOLOGICAL VARIABLES (0 – 3)		
Appetite	<ul style="list-style-type: none"> • The cat is eating normally • The cat is eating more than normal • The cat is eating less than normal • The cat is not interested in food 	0
		1
		2
		3
TOTAL SCORE (0 – 27)		

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307 **Appendix 2:** feline dental chart (Holmstrom S, Frost P and Eisner E. *Veterinary dental techniques: for*
308 *the small animal practitioner*. 2nd ed. W. B. Saunders Company, 1998, pp17-18)

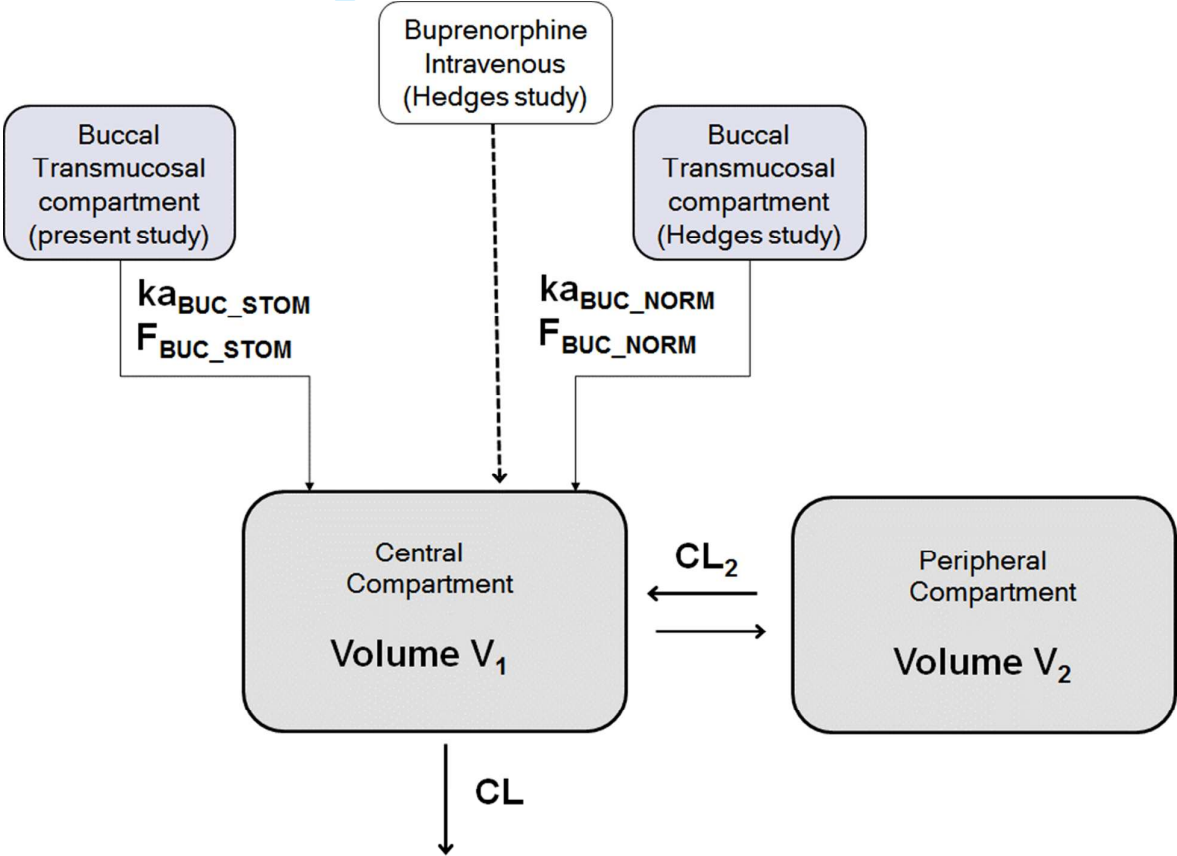
309

Appendix 3: Stomatitis disease activity index score. Dentalvets 2013

STOMATITIS DISEASE ACTIVITY INDEX	0	1	2	3
Owner's evaluation(average appetite/activity/grooming)				
Owner's evaluation perceived comfort				
Maxillary buccal mucosal inflammation				
Mandibular buccal mucosal inflammation				
Maxillary attached gingival inflammation				
Mandibular attached gingival inflammation				
Inflammation lateral to palatoglossal folds				
Molar salivary gland inflammation				
Oropharyngeal inflammation				
Lingual and/or sublingual inflammatio				
Total score(maximum 30)				

Appendix 4: Population pharmacokinetic-pharmacodynamic modelling

A classic two compartment model with first order absorption was the starting point for compartmental modelling of the buccal route. We used the raw data from a previous publication (Hedges et al. 2013 with 6 healthy cats receiving buprenorphine IV and buccally) to support the PK modelling in clinical cats from which only 3 blood samples were taken.



Parameters: CL: body clearance, CL_2 : inter-compartmental clearance, V_1 : central volume of distribution, V_2 : peripheral volume of distribution, ka_{BUC_STOM} : absorption rate constant in cats with stomatitis, ka_{BUC_NORM} : absorption rate constant in normal cats, F_{BUC_STOM} : bioavailability in cats with stomatitis, F_{BUC_NORM} : bioavailability in normal cats.

Goodness of fit:

For each Phoenix NMLE run, plots of goodness of fit were prepared³⁸. The nested candidate models were compared on the basis of their biological plausibility, prediction based diagnostics (PRED, IPRED), residual-type diagnostics (RES and IRES) and numerical diagnostics (minimisation of the Objective Function Value (OVF) statistically tested with the Likelihood Test Ratio (was LRT performed, $\Delta\text{OVF} > 6.64$; $P < 0.01$, $df = 1$, or alternatively use the Akaike Information Criterion, AIC) as well as measures of model stability and adequacy (convergence, precision of the parameters estimates).

Statistical description of the model:

Inter-animal variability was characterised assuming that individual parameters were log-normally distributed around the population typical value (Eq. 1):

$$P_{ij} = \theta_j \times \exp(\eta_{ij}) \quad (1)$$

Where P_{ij} is the j -th parameter value for individual i , θ_j is the typical value for the j -th parameter for the population and η_{ij} is normally distributed around 0 with a variance of ω_j^2 . To minimise the residual variability (difference between predicted and observed values), additive and proportional error models were compared.

Parameters bounded between 0 and 1 (typically bioavailabilities, noted F) were expressed and estimated in the model after a logit transform and the typical value of F (θ_F) was back-converted as in equation 2 to yield final estimate.

$$F_i = \text{inv logit} (\theta_F + \eta_{Fi}) \quad (2)$$

Where F_i is the inverse logit of θ_F , the typical value of the bioavailability, and η_{Fi} is the residual for the i^{th} individual.

The coefficient of variation of the PK parameter was approximated as follows (Eq. 3):

$$CV(\%) = \sqrt{\exp(\omega^2) - 1} \times 100\% \quad (3)$$

Visual predictive checks were built to evaluate the performance of the final model by comparing the median of the simulated ($n=5000$) plasma concentrations with the observed data ($\pm 5^{\text{th}}$ and 95^{th} percentiles).

PK modelling

Base model development for the buccal administration

First, a 2 compartment model was written to fit simultaneously the IV and the buccal route to allow estimation of the physiological PK parameters common to the three routes of administration (namely CL, the total body clearance; V, the volume of the central compartment; CL₂, the intercompartmental clearance and V₂, the volume of the peripheral compartment), as well as the buccal absorption rate constants (k_{abuc}) and the absolute buccal bioavailabilities (F_{buc}). The typical value θ_j and individual η_{ij} were fixed to reduce the number of parameters to estimate in the modelling of the complex SC absorption.

Table1: Comparison of rival models for joint IV and buccal buprenorphine model and selection of best model

Joint model	OFV (-2LL)	AIC	Comment
Combined IV and buccal, proportional error	221	255	Best model
Combined IV and buccal, additional error	443	477	

PK parameters estimates (see also Table 1 in manuscript):

The two routes of administration shared four central PK parameters; clearance (CL = 1.26 L/ kg/ hour, 1.1%), volume of distribution of the central compartment (V₁ = 0.65 L/kg, 0.9%), intercompartmental clearance (CL₂ = 1.19 L /kg/hour, 2.3%) and peripheral volume of distribution (V₂ = 6.96 L/ kg, 7.8%) with a common proportional residual error term.

For PK parameters specific to the buccal treatment, the mean bioavailability in the cats with gingivostomatitis with the current formulation (Buprecare®, animalcare) was 19.5% (IIV 65.7%) compared to 28.8% (IIV 19.6%) in the normal cats in the study by Hedges et al²⁵, in which another formulation was used (Buprenex® Injectable; Reckitt Beckiser Pharmaceuticals). This difference was not significant (P = 0.31). The absorption rate constant in cats with gingivostomatitis was 0.57/hour, yielding an absorption half-life of 1.2 hours. For the normal cats in the study by Hedges et al. ²⁵, the absorption rate constant was 1.39/hour, yielding a significantly shorter absorption half-life of 0.49 hours.

Results and goodness of fit plots:

The goodness of fit figures for the final PK model fitting (buprenorphine and metabolite) are included thereafter:

- Fig suppl. 1: observed values vs population prediction,
- Fig suppl. 2: observed values vs individual predictions,
- Fig suppl. 3: conditional weighted residuals vs time after dose,
- Fig suppl. 4: conditional weighted residuals vs population prediction,
- Fig suppl. 5: individual observed concentrations and model predictions vs time,

Fig suppl. 1 (observed values vs population predictions PRED)

Legends: CObs_A_IV: buprenorphine after IV administration (Hedges et al, 2013), CObs_B_OTM: buprenorphine after buccal administration (Hedges et al, 2013), CObs_C_OTM: buprenorphine after buccal administration (present study), DV = dependent variable (observed value), PRED = population predictions, IPRED = individual predictions

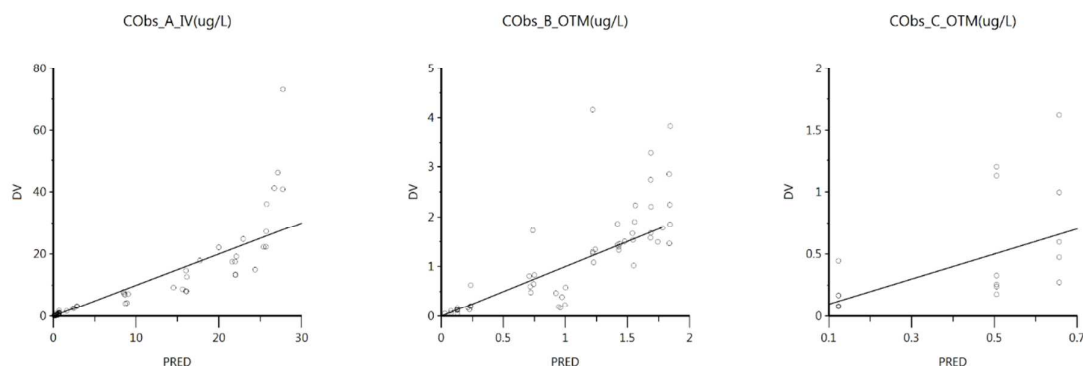
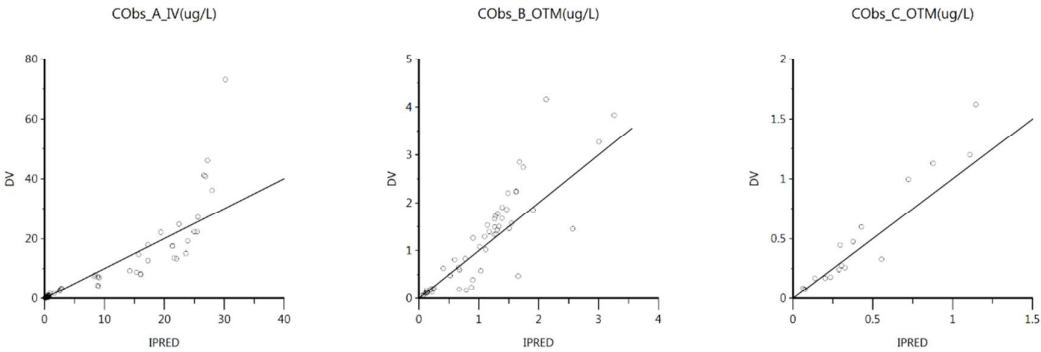


Fig suppl. 2 (observed values vs individual predictions IPRED)

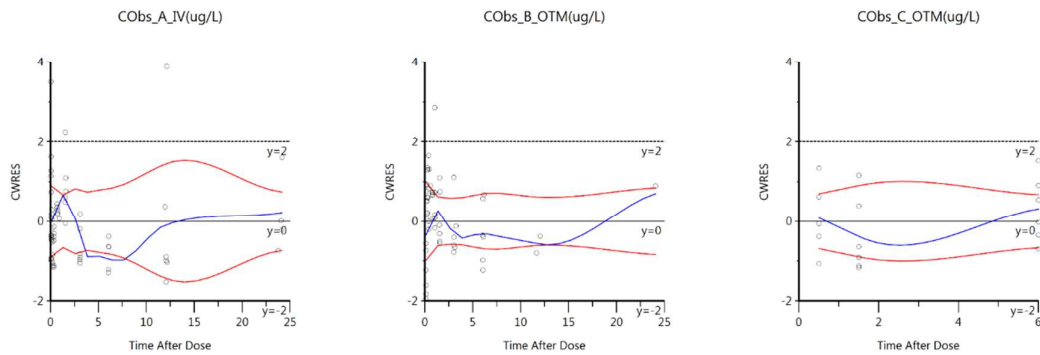


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For Peer Review

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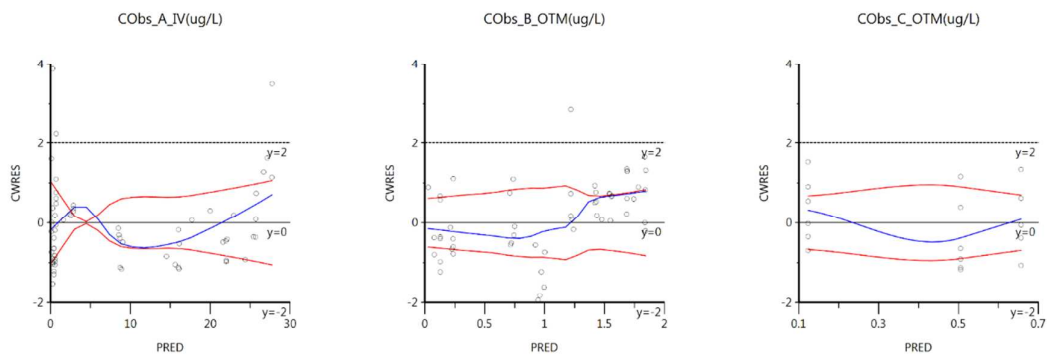
415 Fig suppl. 3 (conditional weighted residuals vs time after dose)



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418 Fig suppl. 4 (conditional weighted residuals vs population prediction)



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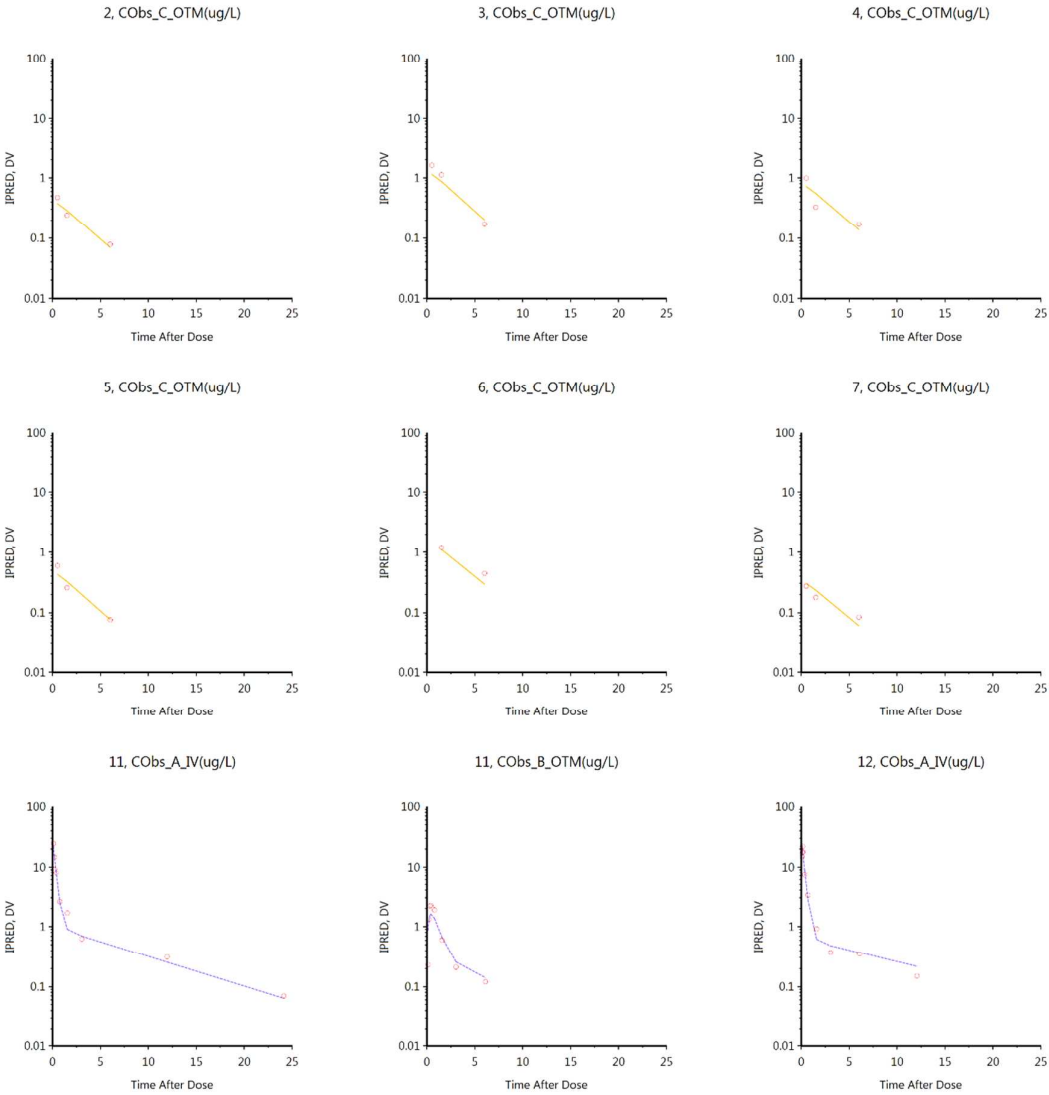
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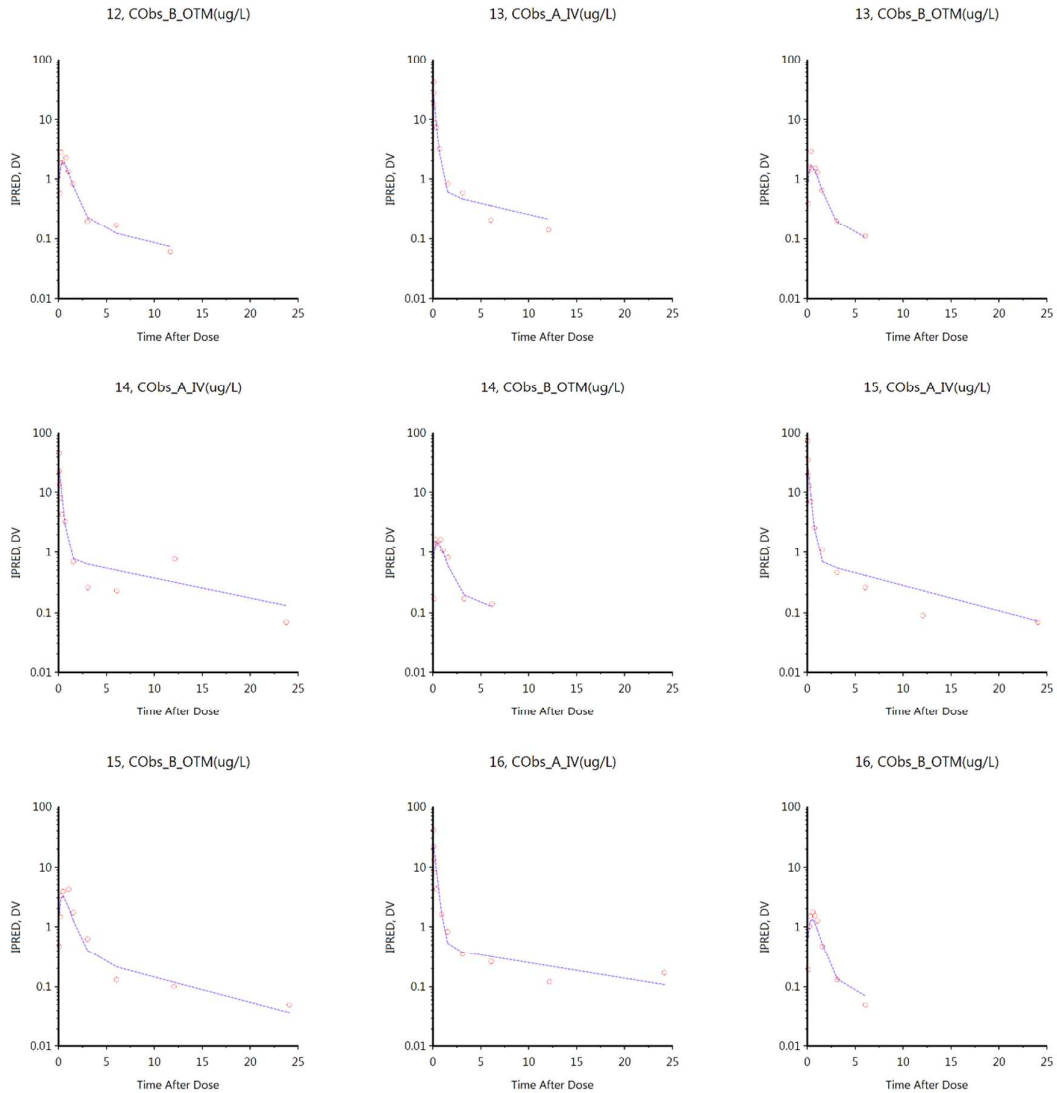
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Fig suppl. 5: Invididual observed concentrations and model predictions vs time. Cats 2 to 7 has gingivostomatitis and were sparsely sampled after administration of buprenorphine 0.02 mg/kg buccally (Formulation: Buprecare, Animalcare). Cats 11 to 16 were normal cats and were densely sampled after administration of 0.02 mg/kg buprenorphine IV (CObs_A) and buccally (Cobs_B) (Formulation: Buprenex, Reckitt Beckiser Pharmaceuticals)





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Conflict of interest

The authors declared no potential conflict of interest for the completion of this study.

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545 *therapeutics*. 2007; 82: 17-20.

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Figure 1: Crossover design of the study for buccal administration of buprenorphine 0.02 mg/kg or saline

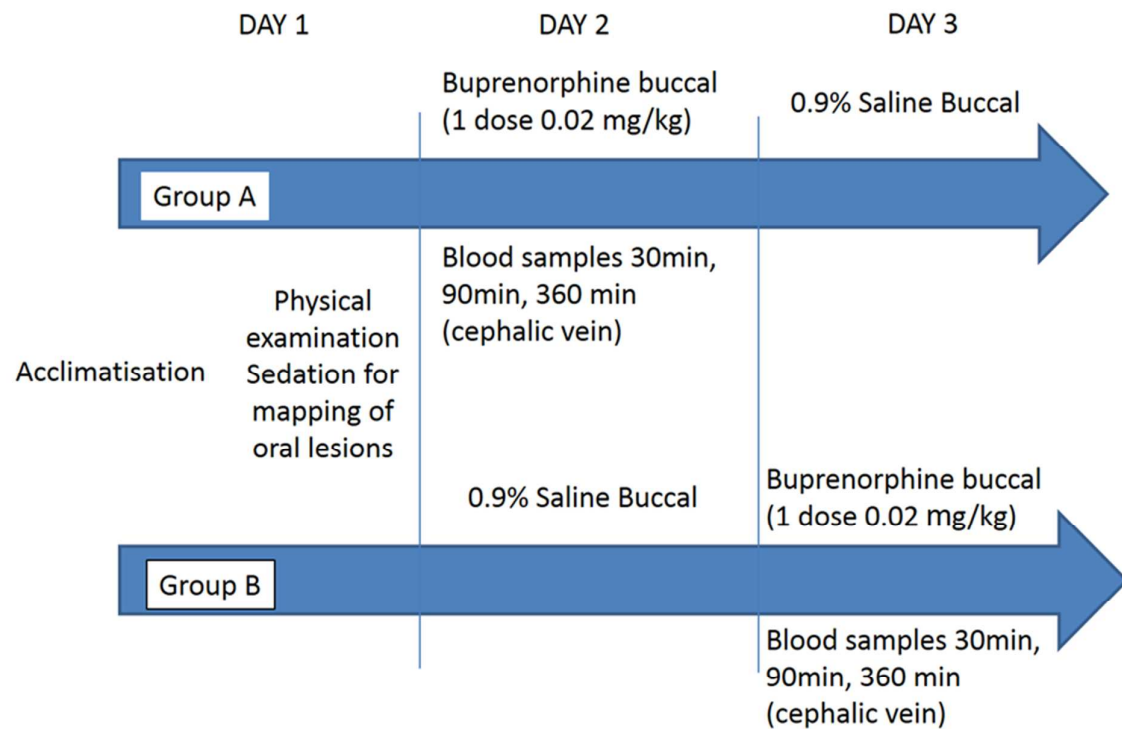


Table 1: Pharmacokinetic parameters estimated to simultaneous population PK modelling of the data from the present study (n = 6 cats, OTM) and data from the Hedges 2013 study (n = 6 cats, IV and OTM)

Parameter	Units	Estimate	IIV (%)
Buprenorphine bioavailability OTM in gingivostomatitis cats (present study)		19.5%	65.7
Buprenorphine Bioavailability OTM in normal cats (Hedges et al 2013)		28.8%	19.6
Clearance	L/kg/h	1.26	1.1
Volume of distribution central compartment	L/kg	0.65	0.9
Intercompartmental clearance	L/kg/h	1.19	2.3
Volume of distribution peripheral compartment	L/kg	6.96	7.8
Absorption rate constant OTM gingivostomatitis cats	1/h	0.573	N.E.
Absorption Half Life OTM gingivostomatitis cats	h	1.2	
Absorption rate constant OTM normal cats	1/h	1.387	N.E.
Absorption Half Life OTM normal cats	h	0.49	
N.E. could not be estimated for individual			

Figure 2 Modified Botucatu pain scores in 6 client-owner cats with gingivostomatitis after receiving buccally saline and buprenorphine OTM (0.02mg/kg). *: $P < 0.05$, Wilcoxon matched -pairs signed rank test

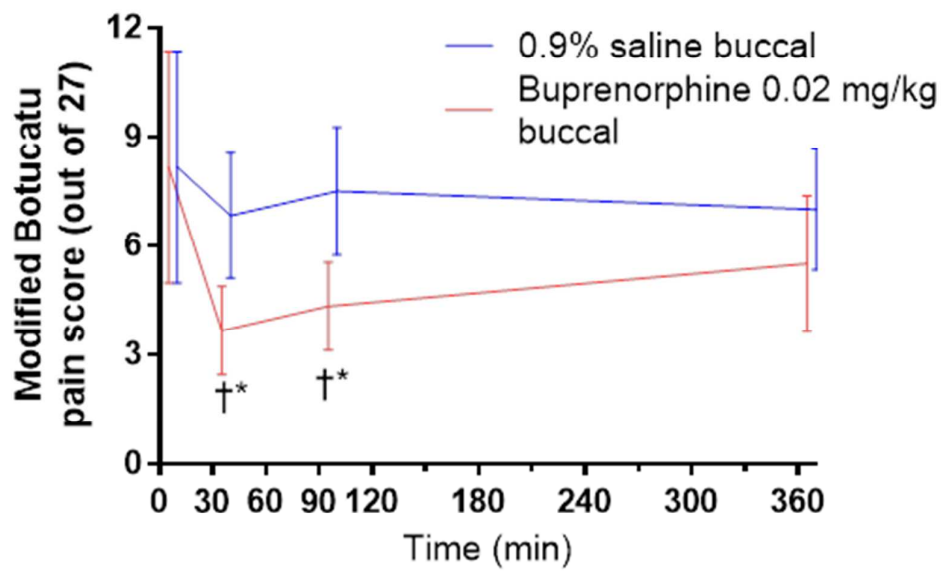
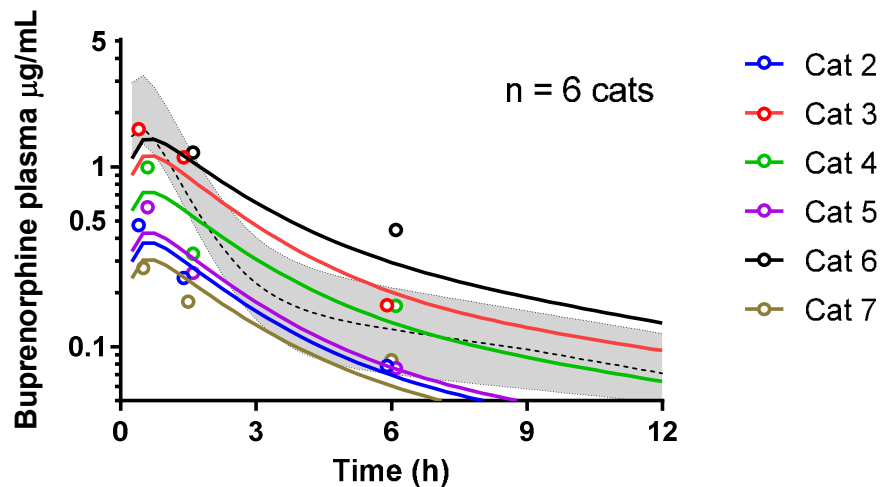


Figure 3: Plot of the buprenorphine concentration ($\mu\text{g/ml}$) in cats with gingivostomatitis and normal cats after receiving 0.02 mg/kg of buprenorphine buccally. For the cats with gingivostomatitis, coloured circles represent the individual measured plasma concentrations and coloured lines the most likely plasma concentration-time profile as informed by the PK model. For reference, the dashed line and grey area represent the median concentration and area between the minimum and maximum plasma concentration observed by Hedges et al. (2014) after administration of the same dose of buprenorphine buccally in healthy cats



Supplementary material

Appendix 1: UNESP-Botucatu Multidimensional Composite Pain Scale for assessing postoperative pain in cats, modified to assess oral pain.

Subscale 1: PAIN EXPRESSION (0 – 12)		
Miscellaneous behaviour	<p>Observe and mark the presence of the behaviours listed below</p> <p>A - The cat is laying down and quiet, but moving its tail</p> <p>B - The cat contracts and extends its thoracic limbs and/or contracts its neck muscles</p> <p>C - The cat's eyes are partially closed (eyes half closed)</p> <p>D - The cat licks and/or bites the surgical wound</p> <p>• All above behaviours are absent</p> <p>• Presence of one of the above behaviours</p> <p><input type="checkbox"/> Presence of two of the above behaviours</p> <p><input type="checkbox"/> Presence of three or all of the above behaviours</p>	<p>A</p> <p>B</p> <p>C</p> <p>D</p> <p>0</p> <p>1</p> <p>2</p> <p>3</p>
Reaction to palpation of the area around the mouth cavity	<p>• The cat does not react when the mouth is touched or pressed;</p> <p>• The cat does not react when the area around the mouth is touched, but does react when it is pressed. It may vocalize and/or try to bite</p> <p>• The cat reacts when the mouth is touched and when pressed. It may vocalize and/or try to bite</p> <p>• The cat reacts when the observer approaches the mouth. It may vocalize and/or try to bite</p> <p>The cat does not allow palpation around mouth cavity</p>	<p>0</p> <p>1</p> <p>2</p> <p>3</p>
Reaction to palpation of the head	<p>• The cat does not react when the head is touched</p> <p>• The cat does not react when the head and neck are touched, but does react when it is pressed. The neck is tense</p> <p>• The cat reacts when the head and neck are touched and when pressed. The neck is tense</p> <p>• The cat reacts when the observer approaches the head It may vocalize and/or try to bite</p> <p>The cat does not allow palpation of the head and neck</p>	<p>0</p> <p>1</p> <p>2</p> <p>3</p>
vocalisation	<p>• The cat is quiet, purring when stimulated, or miaows interacting with the observer, but does not growl, groan, or hiss</p> <p>• The cat purrs spontaneously (without being stimulated or handled by the observer)</p> <p>• The cat growls, howls, or hisses when handled by the observer (when its body position is changed by the observer)</p> <p>• The cat growls, howls, hisses spontaneously (without being stimulated or handled by the observer)</p>	<p>0</p> <p>1</p> <p>2</p> <p>3</p>

Subscale 2: PSYCHOMOTOR CHANGE (0 – 12)		
posture	<ul style="list-style-type: none"> The cat is in a natural posture with relaxed muscles (it moves normally) The cat is in a natural posture but is tense (it moves little or is reluctant to move) The cat is sitting or in sternal recumbency with its back arched and head down; or The cat is in dorso-lateral recumbency with its pelvic limbs extended or contracted <input type="checkbox"/> The cat frequently alters its body position in an attempt to find a comfortable posture 	0 1 2 3
comfort	<ul style="list-style-type: none"> The cat is comfortable, awake or asleep, and interacts when stimulated (it interacts with the observer and/or is interested in its surroundings) The cat is quiet and slightly receptive when stimulated (it interacts little with the observer and/or is not very interested in its surroundings) The cat is quiet and “dissociated from the environment” (even when stimulated it does not interact with the observer and/or has no interest in its surroundings) The cat may be facing the back of the cage <input type="checkbox"/> The cat is uncomfortable, restless (frequently changes its body position), and slightly receptive when stimulated or “dissociated from the environment” The cat may be facing the back of the cage 	0 1 2 3
activity	<ul style="list-style-type: none"> The cat moves normally (it immediately moves when the cage is opened; outside the cage it moves spontaneously when stimulated or handled) The cat moves more than normal (inside the cage it moves continuously from side to side) The cat is quieter than normal (it may hesitate to leave the cage and if removed from the cage tends to return, outside the cage it moves a little after stimulation or handling) <input type="checkbox"/> The cat is reluctant to move (it may hesitate to leave the cage and if removed from the cage tends to return, outside the cage it does not move even when stimulated or handled) 	0 1 2 3
attitude	<p>Observe and mark the presence of the mental states listed below</p> <p>A - Satisfied: The cat is alert and interested in its surroundings (explores its surroundings), friendly and interactive with the observer (plays and/or responds to stimuli) *The cat may initially interact with the observer through games to distract it from the pain. Carefully observe to distinguish between distraction and satisfaction games</p> <p>B - Uninterested: The cat does not interact with the observer (not interested by toys or plays a little; does not respond to calls or strokes from the observer) * In cats, which don't like to play, evaluate interaction with the observer by its response to calls and strokes</p> <p>C - Indifferent: The cat is not interested in its surroundings (it is not curious; it does not explore its surroundings) * The cat can initially be afraid to explore its surroundings. The observer needs to handle the cat and encourage it to move itself (take it out of the cage and/or change its body position)</p> <p>D - Anxious: The cat is frightened (it tries to hide or escape) or nervous (demonstrating impatience and growling, howling, or hissing when stroked and/or handled)</p> <p>E - Aggressive: The cat is aggressive (tries to bite or scratch when stroked or handled)</p> <p><input type="checkbox"/> Presence of the mental state A</p> <p><input type="checkbox"/> Presence of one of the mental states B, C, D, or E</p> <p><input type="checkbox"/> Presence of two of the mental states B, C, D, or E</p> <p><input type="checkbox"/> Presence of three or all of the mental states B, C, D, or E</p>	A B C D E 0 1 2 3

Subscale 3: PHYSIOLOGICAL VARIABLES (0 – 3)		
Appetite	<ul style="list-style-type: none"> • The cat is eating normally • The cat is eating more than normal • The cat is eating less than normal • The cat is not interested in food 	0 1 2 3
TOTAL SCORE (0 – 27)		

Pet Clinic Feline Dental Treatment Chart

M1 109	P4 108	P3 107	P2 106	C1 104	I3 103	I2 102	I1 101	I1 201	I2 202	I3 203	I1 204	P2 206	P3 207	P4 208	M1 209
Right Side								Left Side							
Buccal								Buccal							
Occlusal								Occlusal							
Palatal								Palatal							
Lingual								Lingual							
Occlusal								Occlusal							
Buccal								Buccal							
M1 409	P4 408	P3 407	C1 404	I3 403	I2 402	I1 401	I1 301	I2 302	I3 303	I1 304	P2 307	P3 308	P4 308	M1 309	

Remarks and Diagnosis: _____

Radiographic Evaluation and Assessment: _____

Treatment Summary and Plan: _____

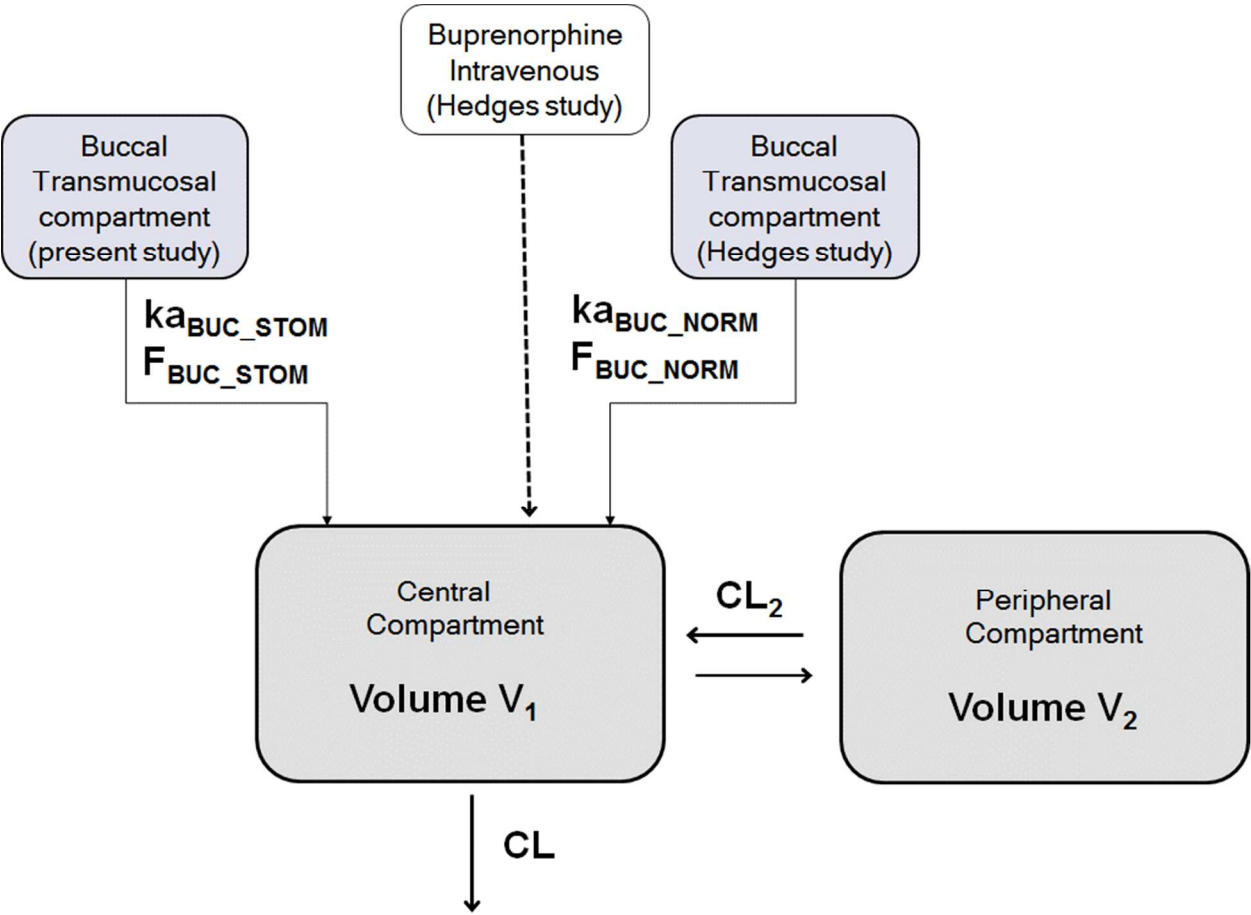
Client Instructions: _____

Appendix 3: Stomatitis disease activity index score. Dentalvets 2013

STOMATITIS DISEASE ACTIVITY INDEX	0	1	2	3
Owner's evaluation(average appetite/activity/grooming)				
Owner's evaluation perceived comfort				
Maxillary buccal mucosal inflammation				
Mandibular buccal mucosal inflammation				
Maxillary attached gingival inflammation				
Mandibular attached gingival inflammation				
Inflammation lateral to palatoglossal folds				
Molar salivary gland inflammation				
Oropharyngeal inflammation				
Lingual and/or sublingual inflammatio				
Total score(maximum 30)				

Appendix 4: Population pharmacokinetic-pharmacodynamic modelling

A classic two compartment model with first order absorption was the starting point for compartmental modelling of the buccal route. We used the raw data from a previous publication (Hedges et al. 2013 with 6 healthy cats receiving buprenorphine IV and buccally) to support the PK modelling in clinical cats from which only 3 blood samples were taken.



Parameters: CL: body clearance, CL_2 : inter-compartmental clearance, V_1 : central volume of distribution, V_2 : peripheral volume of distribution, ka_{BUC_STOM} : absorption rate constant in cats with stomatitis, ka_{BUC_NORM} : absorption rate constant in normal cats, F_{BUC_STOM} : bioavailability in cats with stomatitis, F_{BUC_NORM} : bioavailability in normal cats.

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Inter-animal variability was characterised assuming that individual parameters were log-normally distributed around the population typical value (Eq. 1):

$$P_{ij} = \theta_j \times \exp(\eta_{ij}) \quad (1)$$

Where P_{ij} is the j -th parameter value for individual i , θ_j is the typical value for the j -th parameter for the population and η_{ij} is normally distributed around 0 with a variance of ω_j^2 . To minimise the residual variability (difference between predicted and observed values), additive and proportional error models were compared.

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$$F_i = \text{inv logit} (\theta_F + \eta F_i) \quad (2)$$

Where F_i is the inverse logit of θ_F , the typical value of the bioavailability, and ηF_i is the residual for the i^{th} individual.

The coefficient of variation of the PK parameter was approximated as follows (Eq. 3):

$$CV(\%) = \sqrt{\exp(\omega^2) - 1} \times 100\% \quad (3)$$

Visual predictive checks were built to evaluate the performance of the final model by comparing the median of the simulated (n=5000) plasma concentrations with the observed data (+/- 5th and 95th percentiles).

PK modelling

Base model development for the buccal administration

First, a 2 compartment model was written to fit simultaneously the IV and the buccal route to allow estimation of the physiological PK parameters common to the three routes of administration (namely CL, the total body clearance; V, the volume of the central compartment; CL₂, the intercompartmental clearance and V₂, the volume of the peripheral compartment), as well as the buccal absorption rate constants (ka_{BUC}) and the absolute buccal bioavailabilities (F_{BUC}). The typical value θ_j and individual η_{ij} were fixed to reduce the number of parameters to estimate in the modelling of the complex SC absorption.

Table1: Comparison of rival models for joint IV and buccal buprenorphine model and selection of best model

Joint model	OFV (-2LL)	AIC	Comment
Combined IV and buccal, proportional error	221	255	Best model
Combined IV and buccal, additional error	443	477	

PK parameters estimates (see also Table 1 in manuscript):

The two routes of administration shared four central PK parameters; clearance (CL = 1.26 L/ kg / hour, 1.1%), volume of distribution of the central compartment (V₁ = 0.65 L/kg, 0.9%), intercompartmental clearance (CL₂ = 1.19 L /kg/hour, 2.3%) and peripheral volume of distribution (V₂ = 6.96 L/ kg, 7.8%) with a common proportional residual error term.

For PK parameters specific to the buccal treatment, the mean bioavailability in the cats with gingivostomatitis with the current formulation (Buprecare[®], animalcare) was 19.5% (IIV 65.7%) compared to 28.8% (IIV 19.6%) in the normal cats in the study by Hedges et al ([Hedges, Pypendop et al. 2014](#)), in which another formulation was used (Buprenex[®] Injectable; Reckitt Beckiser Pharmaceuticals). This difference was not significant ($P = 0.31$). The absorption rate constant in cats with gingivostomatitis was 0.57/hour, yielding an absorption half-life of 1.2 hours. For the normal cats in the study by Hedges et al. ([Hedges, Pypendop et al. 2014](#)), the absorption rate constant was 1.39/hour, yielding a significantly shorter absorption half-life of 0.49 hours.

Results and goodness of fit plots:

The goodness of fit figures for the final PK model fitting (buprenorphine and metabolite) are included thereafter:

- Fig suppl. 1: observed values vs population prediction,
- Fig suppl. 2: observed values vs individual predictions,
- Fig suppl. 3: conditional weighted residuals vs time after dose,
- Fig suppl. 4: conditional weighted residuals vs population prediction,
- Fig suppl. 5: individual observed concentrations and model predictions vs time,

Fig suppl. 1 (observed values vs population predictions PRED)

Legends: CObs_A_IV: buprenorphine after IV administration (Hedges et al, 2013), CObs_B_OTM: buprenorphine after buccal administration (Hedges et al, 2013), CObs_C_OTM: buprenorphine after buccal administration (present study), DV = dependent variable (observed value), PRED = population predictions, IPRED = individual predictions

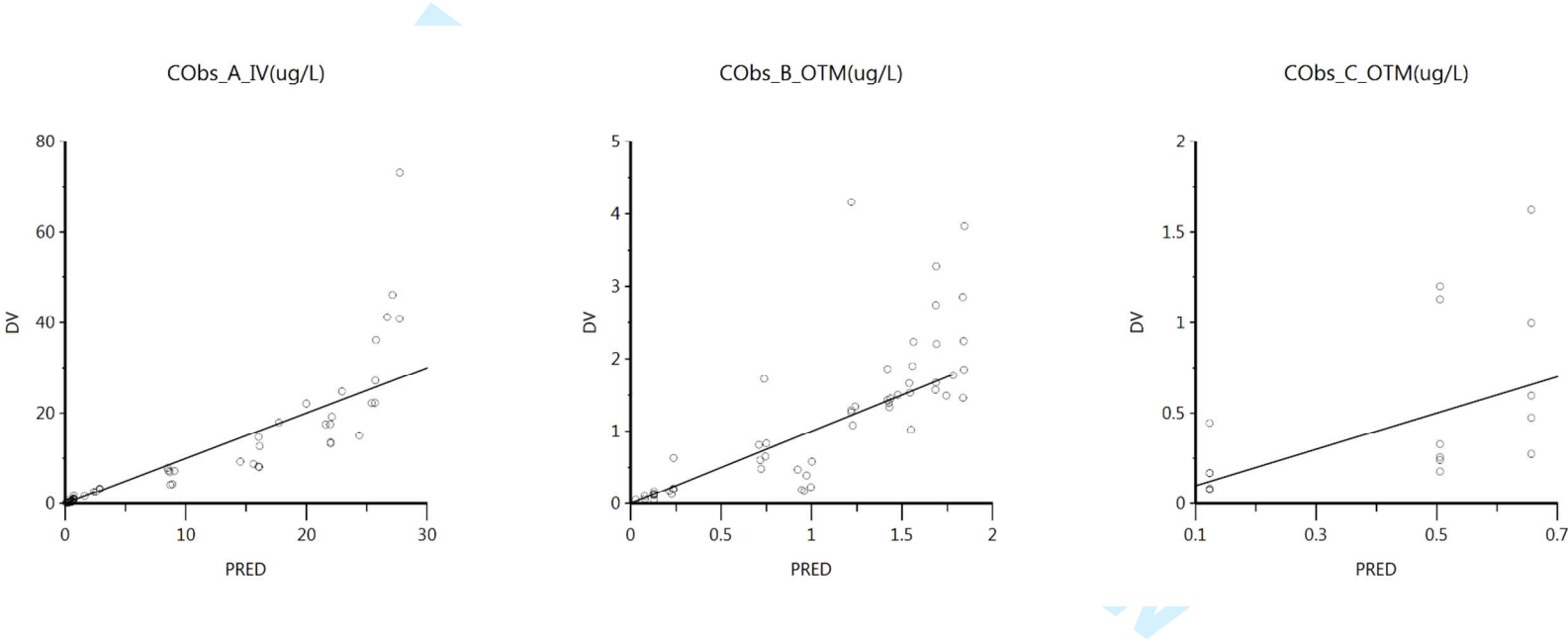


Fig suppl. 2 (observed values vs individual predictions IPRED)

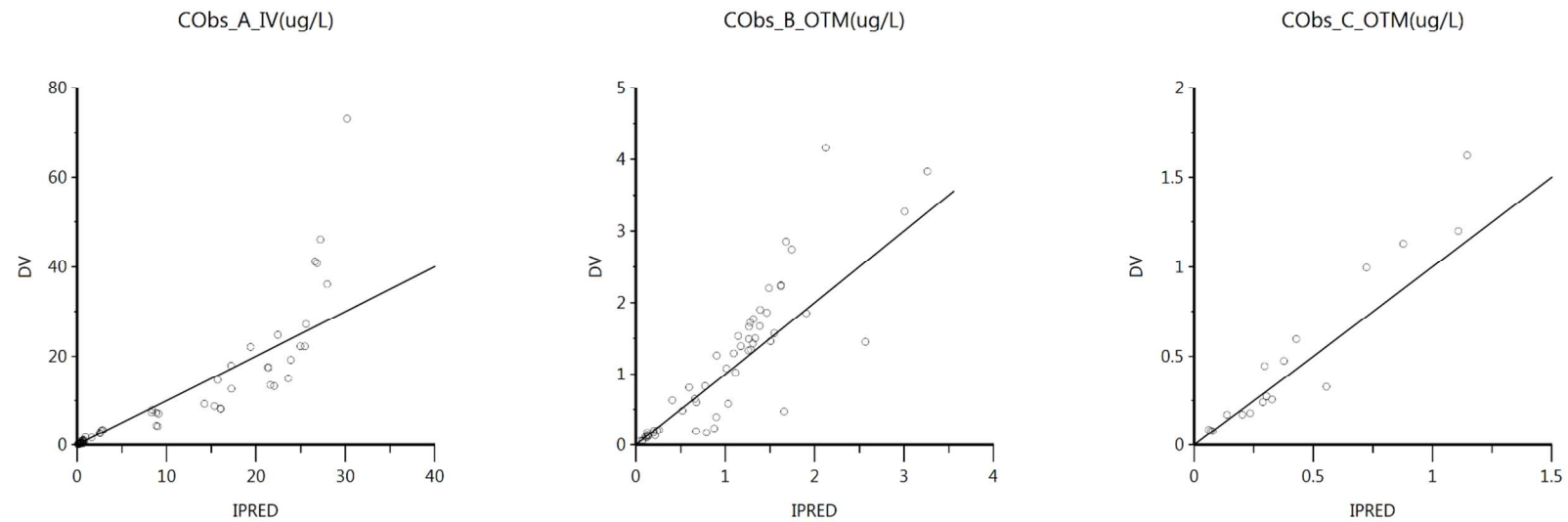


Fig suppl. 3 (conditional weighted residuals vs time after dose)

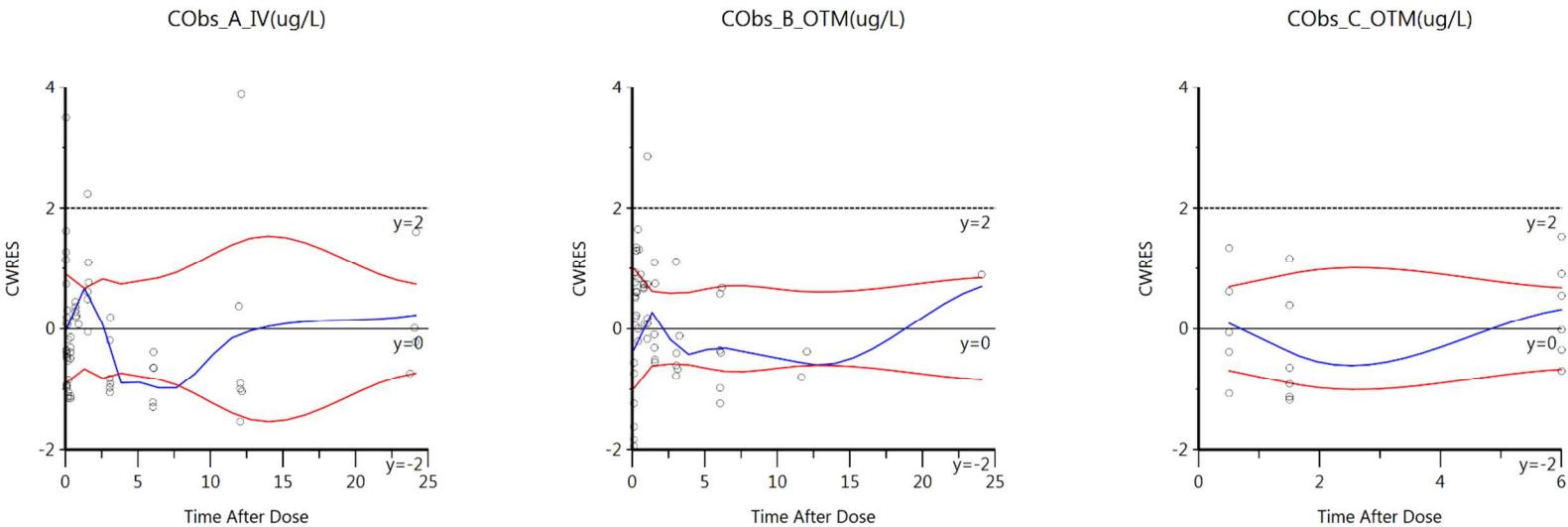


Fig suppl. 4 (conditional weighted residuals vs population prediction)

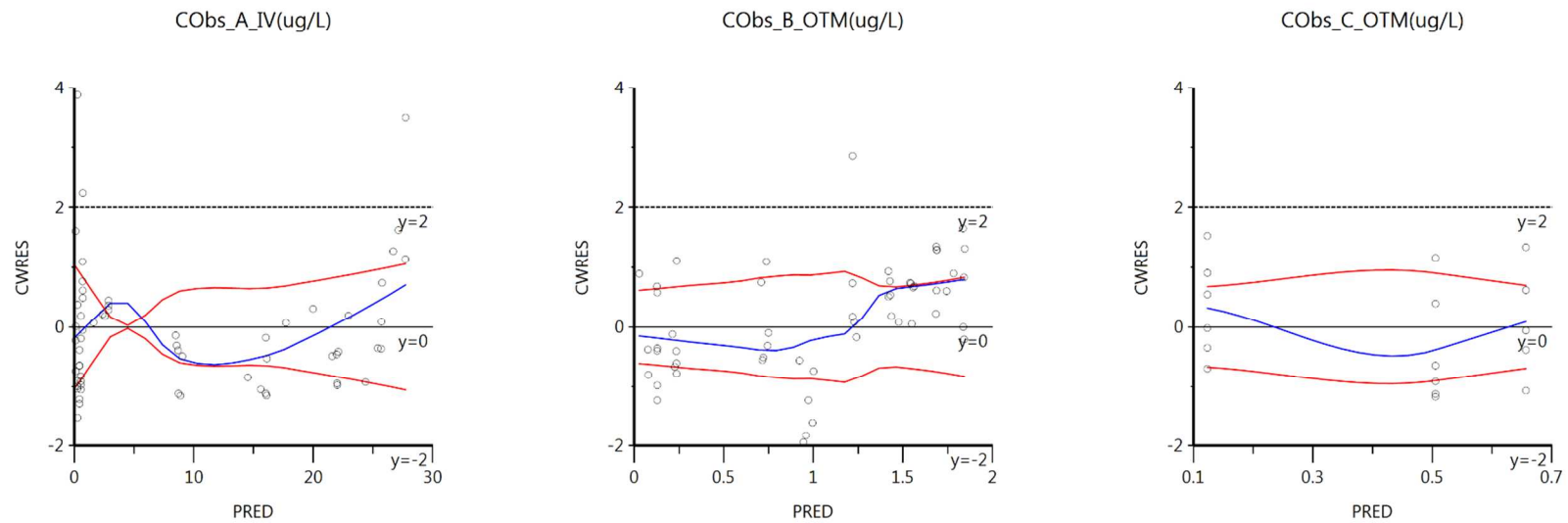


Fig suppl. 5: Invididual observed concentrations and model predictions vs time. Cats 2 to 7 has gingivostomatitis and were sparsely sampled after administration of buprenorphine 0.02 mg/kg buccally (Formulation: Buprecare, Animalcare). Cats 11 to 16 were normal cats and were densely sampled after administration of 0.02 mg/kg buprenorphine IV (CObs_A) and buccally (Cobs_B) (Formulation: Buprenex, Reckitt Beckiser Pharmaceuticals)

